

AD-A280 204



AD _____

CONTRACT NO: DAMD17-87-C-7014

**TITLE: PRODUCTION OF ANTIGENS AND ANTIBODIES FOR DIAGNOSIS
OF ARBOVIRUS DISEASES**

PRINCIPAL INVESTIGATOR: Robert E. Shope, M.D.

**CONTRACTING ORGANIZATION: Yale University School of Medicine
333 Cedar Street
P.O. Box 20846
New Haven, Connecticut 06510-8047**

REPORT DATE: May 20, 1994

TYPE OF REPORT: Annual Report

**PREPARED FOR: U.S. Army Medical Research, Development,
Acquisition and Logistics Command (Provisional),
Fort Detrick, Frederick, Maryland 21702-5012**

**DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited**

**The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.**

94-17976



WAB

DTIC QUALITY INSPECTED 1

94 6 10 081

①

**DTIC
ELECTE
JUN 13 1994
S G D**

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
<small>Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.</small>				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 20 May 1994	3. REPORT TYPE AND DATES COVERED Annual Report (4/1/90 - 3/31/91)		
4. TITLE AND SUBTITLE Production of Antigens and Antibodies for Diagnosis of Arbovirus Diseases		5. FUNDING NUMBERS Contract No. DAMD17-87-C-7014		
6. AUTHOR(S) Robert E. Shope, M.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Yale University School of Medicine 333 Cedar Street P.O. Box 20846 New Haven, Connecticut 06510-8047		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research, Development, Acquisition and Logistics Command (Provisional), Fort Detrick, Frederick, Maryland 21702-5012		10. SPONSORING/MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited		12b. DISTRIBUTION CODE		
13. ABSTRACT (Maximum 200 words) <p>Antigens and antibodies were produced and standardized for use in ELISA. Antigens were produced by sucrose-acetone extraction of suckling mouse brain for 14 arboviruses and residual infectivity was inactivated with beta-propiolactone. An additional 10 viruses were passaged in mice and the mice were stored frozen awaiting sucrose-acetone extraction of the brains.</p> <p>Rabbits were immunized successfully intravenously with West Nile and Sindbis immunogens grown in RK-13 rabbit cells. ELISA testing of the resulting ammonium sulfate precipitated IgG from West Nile, Sindbis, and other rabbits immunized in past contract periods showed generally excellent titers.</p>				
14. SUBJECT TERMS		15. NUMBER OF PAGES		
		16. PRICE CODE		
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

SUMMARY

Antigens and antibodies were produced and standardized for use in ELISA. Antigens were produced by sucrose-acetone extraction of suckling mouse brain for 14 arboviruses and residual infectivity was inactivated with beta-propiolactone. An additional 10 viruses were passaged in mice and the mice were stored frozen awaiting sucrose-acetone extraction of the brains.

Rabbits were immunized successfully intravenously with West Nile and Sindbis immunogens grown in RK-13 rabbit cells. ELISA testing of the resulting ammonium sulfate precipitated IgG from West Nile, Sindbis, and other rabbits immunized in past contract periods showed generally excellent titers.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

In conducting research using animals, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Robert E. Shope *May 20, 1984*
PI Signature Date

Accession For	
NTIS	CRA&I <input checked="" type="checkbox"/>
DTIC	TAB <input type="checkbox"/>
Unannounced <input type="checkbox"/>	
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and/or Special
A7	

TABLE OF CONTENTS

Front page.....	1
Report documentation page.....	2
Summary.....	3
Foreword.....	3
Table of contents.....	4
Body of report.....	5
1. Production of mouse brain sucrose-acetone extracted antigens.....	5
2. Production of antibody to arboviruses in rabbits.....	6
Discussion and conclusions.....	6

BODY OF REPORT

1. Production of mouse brain sucrose-acetone extracted antigens.

Fourteen antigens were prepared during the project extension as listed here:

Antigen	Strain	Passage	Number of lots	Volume (ml)	Volume to date
Bandia	RV611	sm8	1	67	284
Dugbe	IbAr1792	sm12	3	154	259
LaCrosse	prototype	sm8V1sm1	1	13	13
Maguari	BeAr7272	sm9	1	45	268
Oriboca	BeAn17	sm14	1	23	23
Qalyub	EgAr 370	sm4	1	48	277
Rocio	SPH34675	sm6	2	87	87
Salehabad	I81	sm16V2	1	62	85
Sicilian SF	Sabin	sm37V2	1	14	37
Sindbis	EgAr339	sm3V1sm1	1	40	40
tick-borne enc. Czech		sm6	4	200	200
West Nile	Eg 101	sm13V1sm2	1	25	25
Toscana	ISS Ph1-3	V1sm2	1	35	314
VS-New Jersey	Hazelhurst	CE18V4sm1	1	58	422

Additionally, 10 viruses were passaged in baby mice awaiting preparation of antigen lots. These were Bangui (6 lots), Candiru (6 lots), Colorado tick fever (2 lots), Guaroa (3 lots), Inkoo (5 lots), Sagiya (3 lots), Sindbis (4 lots), Tensaw (3 lots), West Nile (5 lots), and Zika (1 lot).

2. Production of antibody to arboviruses in rabbits.

Rabbits were immunized with West Nile and Sindbis viruses during this contract period. Results of ELISA with ammonium sulfate concentrates with these and IgG from other rabbits immunized in the last contract period were:

Virus	Optimal titer in ELISA	Volume (ml)
Cocal	1:8000	6
	1:16000	80
	1:32000	96
Jamestown Canyon	1:16000	62
	1:32000	30
Mucambo	1:400	13
	1:500	76
	1:4000	27
	1:32000	40
Semliki Forest	1:32000	35
	1:64000	40
Sindbis	1:4000	30
	1:16000	30
	1:32000	38
Snowshoe hare	1:500	73
West Nile	1:32000	15
	1:64000	40
	1:128000	50

Antigens for the above tests were employed at 1:10.

DISCUSSION AND CONCLUSIONS

The problem of adapting some of the arboviruses to growth in rabbit kidney cells (RK-13) was not solved. In spite of this, for those arboviruses that did adapt, this system (infected rabbit kidney cells as immunogens for rabbits) with boosting at least 2 months after the primary series of inoculations, functioned well to yield high-titered IgG. In use with sucrose-acetone extracted mouse brain antigens, the IgG provided many excellent antigen-antibody sets for use in the ELISA, and is adaptable to rapid and sensitive diagnosis in military field laboratories.